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# Can floral volatile organic compounds contribute to the taxonomy of the *Rhamnus* sect. *Alaternus*?

Leonardo Llorens[1], Pere Ferriol[1], Joan Tomàs[2], María Trinidad García[3], Lorenzo Gil[1]

**1** *Interdisciplinary Ecology Group, Department of Biology, University of the Balearic Islands (UIB), Ctra, Palma-Valldemossa Km. 7,5, E-07122 Palma de Mallorca, Balearic Islands, Spain* **2** *Department of Biology (Botany), University of the Balearic Islands (UIB), Ctra. Palma-Valldemossa Km. 7,5, E-07122 Palma de Mallorca, Balearic Islands, Spain* **3** *Scientific and Technical Services, University of the Balearic Islands (UIB), Carretera de Valldemossa Km. 7,5, E-07122 Palma de Mallorca, Balearic Islands, Spain*

Corresponding author: Leonardo Llorens (lleonard.llorens@gmail.com)

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## Abstract

The chemistry of plants represents a taxonomic and phylogenetic value. Researchers have recently used volatile organic compounds (VOCs) for taxonomic studies. The present work analysed and determined, for the first time, the usefulness of floral volatile compounds in the taxonomy of two species of *Rhamnus* sect. *Alaternus*, as well as the hybrid between these taxa. The two species show significant quantitative and qualitative differences. The terpenes linalool, ocimene, caryophyllene, and green volatiles are exclusive to *Rh. alaternus*, while farnesene (terpene), methyl salicylate and methyl benzoate (benzoids) are obtained from *Rh. ludovici-salvatoris*. Both volatilomes were attractive to Hymenoptera pollinators; therefore, these pollinators could serve as the main hybridisation vector. In addition, *Rh. alaternus* shows greater chemical and genetic heterogeneity than *Rh. ludovici-salvatoris*. Hybrids between the two species, *Rh.×bermejoi*, are closer in chemical similarity to *Rh. alaternus* than *Rh. ludovici-salvatoris*.

**Keywords**

Balearic Islands, hybrids, *Rhamnus*, taxonomy, VOCs

## Introduction

Plant chemistry has repeatedly been used in systematics (Hegnauer 1986a; Tundis et al. 2014 It has long been known that chemical traits and characteristics, notably the secondary metabolites (such as alkaloids, terpenoids, and flavonoids) contained in vegetative parts of the plants, help to establish relationships between plant taxa (Cronquist

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1977; Singh R 2016). Moreover, they have wide applications in plant systematics (see Seigler 1981; Roma-Marzio et al. 2017); however, their use presents some limitations (Hegnauer 1986b; Hadacek 2002). It is also known that the variability of volatile secondary metabolites in the vegetative parts of plants can be high (Ahmad et al. 2014). For this reason, several authors consider the construction of phylogenies on this basis too labile (Whitehead and Peakall 2009). The chemistry of floral biogenic volatile organic compounds (BVOCs) has been well studied as it relates to communication (with pollinators) and defence (mainly herbivore-related) (Bouwmeester et al. 2019). The analysis of BVOCs also enables the evaluation of their important role in the adaptation, environmental adequacy, and medicinal interest of plants (Goswami et al. 2016).Their stability and repeatability can also allow accurate identification of natural or cultivated closely-related species and varieties (Nogueira et al. 2001; Kong et al. 2012; Yang et al. 2014; Carta et al. 2015; Jaeger et al. 2016; Peruzzi et al. 2019). Recently, BVOCs have also been studied from the standpoint of taxonomy (e.g., Levin et al. 2003; Raguso et al. 2006; Meekijjaroenroj et al. 2007; Feulner et al. 2011; Tóth et al. 2016; Stocki et al. 2020), showing their utility as discriminating characteristics. BVOCs have also been used to recognise hybrids, in which new compounds, absent in the parents, can appear (Orians 2000; Cheng et al. 2011), together with others that are not taxonomically relevant (Georgescu et al. 2015). Additionally, some VOC-related phylogenetic patterns have been recognised in some taxa, such as *Ophrys* (Gögler et al. 2009).

*Rhamnus* is a wide-ranging genus of the Temperate and (sub)Tropical regions of the Northern Hemisphere that comprises more than 100 species (Hauenschild et al. 2016). In the Mediterranean region it is represented by 37 species, where *Rh. cathartica* L. and *Rh. alaternus* L. are the most widespread (Henning and Raab-Straube 2016). Molecular studies performed on *Rhamnus*, excluding *Oreoherzogia* W.Vent (Hauenschild et al. 2016), recognise the existence of at least two sections: sect. *Rhamnus* and sect. *Alaternus* (Mill.) DC. The latter includes different species of Mediterranean and Macaronesian distribution (such as *Rh. alaternus* L. and related taxa, *Rh. lojaconoi* Raimondo, *R. ludovici-salvatoris* Chodat for Mediterranean, as well *Rh. glandulosa* Ait. and *Rh. integrifolia* DC. for Macaronesia. Generally, hybrids are uncommon or rare; however, *Rh. cathartica* (Gil-ad and Reznicek 1997; Kurylo et al. 2007) and, occasionally, *Rh. alaternus* (Llorens 1979; Fraga and Bermejo 2008) have shown a notable capacity to hybridise.

*Rhamnus alaternus* is an evergreen tree native to the Mediterranean region and is often cultivated as an ornamental garden shrub in Mediterranean-climate regions. For this purpose, this tree was introduced to many areas of the Australasian-Pacific region, where it became an invasive tree along coastlines (Muyt 2001; GISD 2018). This wide distribution and the different types of plant communities in which it thrives agree with its remarkable morphological variability (López-González 2006; Rivas-Martínez and Pizarro 2011, 2013). On the other hand, although *Rh. alaternus* is a defined species, its differentiation from other species of the *Rh.* sect. *Alaternus*, especially the hybrids, is problematic (Ferriol et al. 2009). This differentiation occurs with *Rh. ludovici-salvatoris* (Fig. 1), from which it differs basically in its larger size and the arrangement of the leaf teeth (approximately perpendicular to the margin in *Rh. ludovici-salvatoris* and obliquely oriented upwards in

*Rh. alaternus*). This difficulty has taxonomic and nomenclatural consequences, especially in plants that develop in unfavorable conditions. This difficulty occurs with the definition and identification of some specimens, such as *Rh. jacobi-salvadorii* O.Bolòs & Vigo or *Rh. alaternus* var. *ferruginea* Pourr. ex Texidor, making it difficult to establish a more precise distribution range. This is the case, for example, in the confusing or erroneous indications of *Rh. ludovici-salvatoris* in the eastern Iberian Peninsula or the actual distribution of the hybrid of this species with *Rh. alaternus* (i.e., *Rh.* ×*bermejoi* Fraga & Rosselló).

The Balearic Islands are the largest archipelago of continental islands in Spain. These islands display a complex palaeogeography, although they have been isolated relatively recently. The eastern islands (Gymnesian) and western islands (Pithyusian) have been separate groups from the early Pliocene. Nevertheless, the Gymnesian Islands (Majorca and Minorca) have only been completely isolated from each other since the Würm glaciation, approx. 15,000 ya (Cardona and Contandriopoulos 1979). In these islands, only two species from *Rh.* sect. *Alaternus* grow: the Mediterranean *Rh. alaternus* and the Gymnesian endemic *Rh. ludovici-salvatoris*. However, both species are related, and some hybrid plants have been identified in one locality of Minorca (Tirant) (Llorens 1979; Fraga and Rosselló 2008). Despite this, phenological and ecological barriers establish good isolation of the populations (Ferriol et al. 2009).

The chemical profile of the floral scent of the taxa of *Rhamnus* sect. *Alaternus* present in Majorca, Minorca, Ibiza, and eastern Iberian Peninsula was analysed in order to address the following three goals: 1) to recognise the volatile compounds of the two species, 2) to establish analogies and chemical differences between the hybrid plants and the parental species, and 3) to determine their value in taxonomy and their usefulness as discriminating characteristics in these species and their hybrids, as well as to establish their possible application to the taxonomy of other *Rhamnus* species.

## Materials and methods

### Plant material

The two species under study and their hybrids were collected from the Balearic Islands and the eastern Iberian Peninsula (Fig. 2, Suppl. material 1). Samples of natural hybrid plants reported as *Rh.* ×*bermejoi* (Llorens 1979; Fraga and Rosselló 2008) were collected in the locality of Tirant (N of Minorca), where they coexist with the parental species. Hybrid plants obtained by manual artificial hybridisation, cultivated and vegetatively propagated were also sampled. A total of 35 plants (14 female and 21 male) were screened for floral VOCs. The wild plants were collected from 13 sites, and the cultivated hybrids were collected from two sites.

### Floral scent trapping

The volatiles emitted by flowers were obtained by the headspace solid phase extraction (HS-SPME) sampling technique, adapted from Friberg et al. (2013) and Tomas et al.

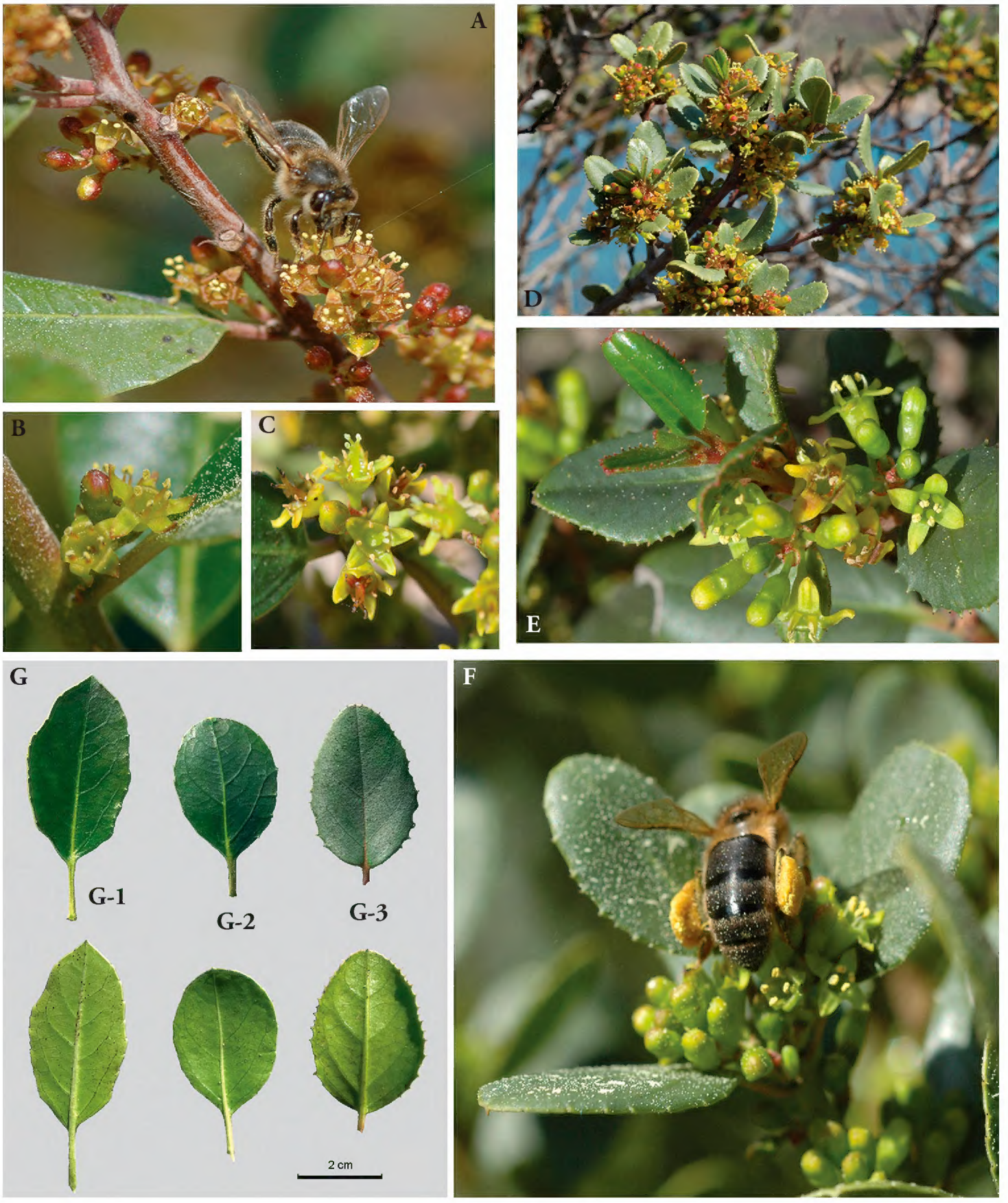


**Figure 1.** Inflorescences, flowers and major pollinator (*Apis mellifera*) of *Rhamnus* **A–C** *Rh. alaternus* **D, E** *Rh. ludovici-salvatoris* **F** *Rh.* ×*bermejoi* **G** leaves of the three species.

(2022). Volatile compounds were extracted in a manual SPME holder together with 10 mL vials and PDMS-DVD fibres (Supelco Inc., Bellefonte, USA). First, ten flowers of each plant were placed in a vial. Next, these vials were placed at 25 °C for 20 min; then, the SPME fibre was exposed in the upper space (headspace) of the sealed vial for 30 min at 25 °C to adsorb the analytes. Three collections and their respective controls (empty vials) were prepared simultaneously. In addition, to distinguish between

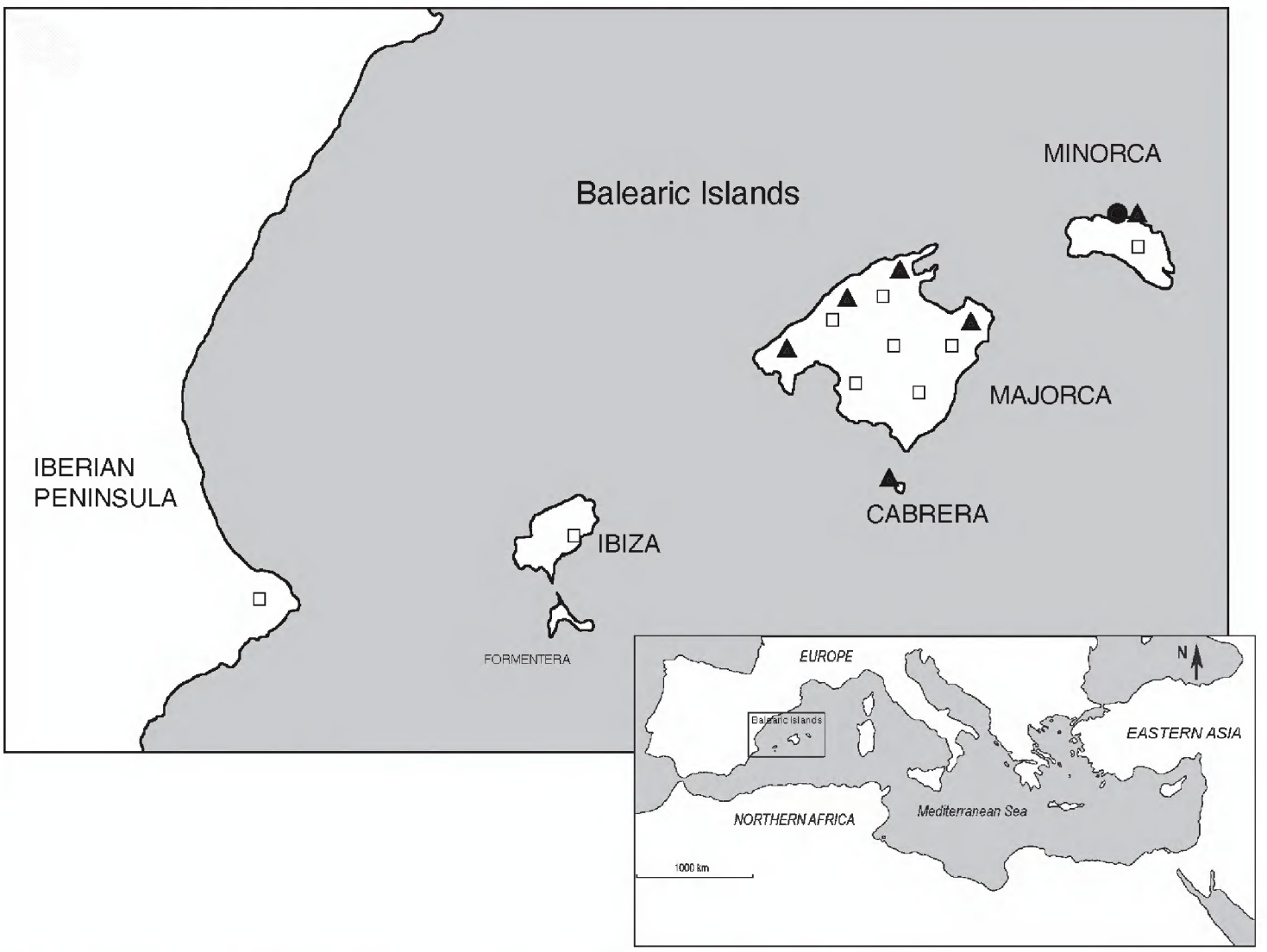


**Figure 2.** Location of the *Rhamnus* populations included in the study. (See Suppl. material 1 for population abbreviations). *Rh. alaternus* open squares; *Rh. ludovici-salvatoris* filled triangles; Hybrids filled circle.

BVOCs from flowers and those from flower pedicels, volatiles emitted by ten pedicels in both parental species were analysed.

## Evaluation of scent composition

Analyses were conducted no longer than 24 hours after collection. HS-SPME-GC-MS analyses were performed on an Agilent 6980 GC - MDS 5975 inert XL (Agilent Technologies, USA), using a Supelcowax 10 gas capillary column (60 m × 0.25 mm × 0.25 mm; Supelco, Bellefonte, Pennsylvania, USA). The PDMS-DVB fibre of the SPME was aged for 3 min in the inlet of the GC. Helium was used as a carrier gas at a flow of 1.3 mL $min^{-1}$. The splitless sample injection mode was used. The injector temperature was 220 °C. The initial oven temperature was programmed as follows: 45 °C for 2 min, increasing to 250 °C at a rate of 5 °C $min^{-1}$, which was continued for 5 min. These settings were sufficient for the quantitative desorption of all analytes studied. Mass spectra (MS) were obtained in electron ionisation (EI) mode at 70 eV, while the ionisation source was 180 °C. The MS scan range was set between 45–300 amu. The chromatograms and spectra of the samples were processed using the GC-EM software Turbomass version 5.1 (Perkin-Elmer, Inc.).

The main isolated volatile compounds were annotated by comparing the mass spectra with mass spectral libraries (Wiley 7th edition and NIST08) and by comparing the calculated retention indices with those provided by NIST08, Adams (2007) and El-Sayed (2012). The annotation of the main compounds was verified using an in-house-developed mass spectra/RI library. The main compounds were verified by comparison with synthetic standards. All compounds that were not identified immediately as floral/plant volatiles were verified in the literature to determine if they were accepted as floral BVOCs or at least as potential volatiles.

### Statistical analysis of data

Differences in BVOCs composition were analysed using the statistical software R version 4.1.2 (R Project for Statistical Computing). Since the total amount of volatiles released strongly fluctuated between individuals, we used the relative amounts taken as the peak area of each compound in relation to the total peak area. Those compounds considered as likely artefacts were excluded from the analysis. The volatiles from three plants were analysed for each population, averaged, and then treated as a single data set.

Data were transformed using the *decostand* function in the *vegan* library (Oksanen et al. 2019). To assess similarities between individual samples, a permutational multivariate analysis of variance (PERMANOVA) was performed with the *adonis2* function on the *vegan* library using the Bray-Curtis dissimilarity indices (Oksanen et al. 2019). Finally, as a post hoc test to detect differences in volatile composition between each of the species, a pairwise contrast for multilevel comparison (*pairwise.adonis* function, Martínez-Arbizu 2020) was used.

Significant *p*-values in a PERMANOVA indicate a significant difference between groups in the centroid or spread of objects in a multivariate space, so non-metric multidimensional scaling (NMDS, metaMDS function with Bray-Curtis distance, performing a Wisconsin double standardisation and also an sqrt transformation) was used. (Clarke et al. 2014).

To test whether there is a separation between groups in relation to the sex of the flowers, an analysis of similarities (*anosim* function, *vegan* package) was performed. To identify the percentage contributions of each volatile compound to the average dissimilarity amongst the three Rhamnus species, the similarity percentage analysis (SIMPER, the simper function in vegan) was used (Clarke 1993).

## Results

### Floral scent chemistry

No relevant traces of BVOCs were detected in floral pedicel samples. In the flowers, the entire GC–MS dataset allowed the detection of 41 compounds, of which 22 had greater presence and abundance (with percentages higher than 0.1%; Suppl. material

2). Chromatographic profiles of *R. alaternus* showed high heterogeneity. Thus, some plants exhibited the presence of certain components in high proportions (up to 89.9%), e.g., ionones (dihydro-β-ionone and α-ionone), 3-hexen-1-ol, acetate (*Z*), benzaldehyde 4-dimethoxy, caryophyllene, and linalools (cis-linaloxide, (*E*)-linalool oxide (furanoid), linalool, hotrienol). However, some of these components were absent in other plants. By contrast, despite including a larger number of samples, *R. ludovici-salvatoris* showed a remarkable uniformity. Thus, the major compounds, such as methyl salicylate, methyl benzoate, or α-farnesene, were found in 85–100% of the samples. Similarly, the largest number of minority compounds, such as ethyl benzoate, 1-butanol, and 3-methyl-benzoate, were present in more than 75% of the samples.

### Floral scent species relations

The chromatographic profiles of *Rh. alaternus* and *Rh. ludovici-salvatoris* showed significant qualitative and quantitative differences between the species (Suppl. material 2 and Fig. 3). Thus, the following components were exclusive to *Rh. alaternus*: acetic acid hexyl ester, certain terpenoids and ocimene including *cis*- and *trans*-β-ocimene (6.73%); linalools including linalool, cis-linaloxide, trans-linalool oxide furanoid, linalyl formate, and hotrienol (9.10%); caryophyllenes including β- and α-caryophyllene (15.71%), and ionones including α-ionone and dihydro-β-ionone (48.28%). On the other hand, in *Rh. ludovici-salvatoris*, unique compounds were found, namely benzoic acid derivatives (methyl salicylate, methyl benzoate, 1-butanol, 3-methyl benzoate) and farnesenes (farnesene and α-farnesene). Among the quantitatively differential products, 3-hexen-1-ol acetate (Z) emerged because in *Rh. alaternus*, it was present in high amounts (25.92 ± 7.55%), while in *Rh. ludovici-salvatoris* it was only found in low quantities (0.65 ± 0.37%) and in less than half of the samples.

The results of the SIMPER analysis (Table 1 and Fig. 4) clearly highlight the differences in the aroma profile of the two species. They show that the two species have a distinct floral aroma profile and that four compounds (methyl salicylate, 3-hexen-1-ol acetate, ionone complex, and b-caryophyllene) account for 75% of these differences. On the other hand, no differences were found between the BVOC profiles in relation to sex (ANOSIM statistic R = -0.03533, p = 0.8222). According to the chemical composition of the hybrid samples and their uniformity, it is noteworthy that they exhibited a great affinity with *Rh. alaternus* (Table 1, Figs 3, 4, Suppl. material 2).

## Discussion

Volatile compounds of the green parts of the plants have been used repeatedly as taxonomic characteristics (see Hegnauer 1986a, Nwafor et al. 2018). By contrast, the analysis of volatile biogenic compounds of flower aromas (floral BVOCs) has been scarcely studied. Despite this, most recently, the qualitative and quantitative variations of these compounds have shown their value as discriminating characteristics in related species or in ecotypes,

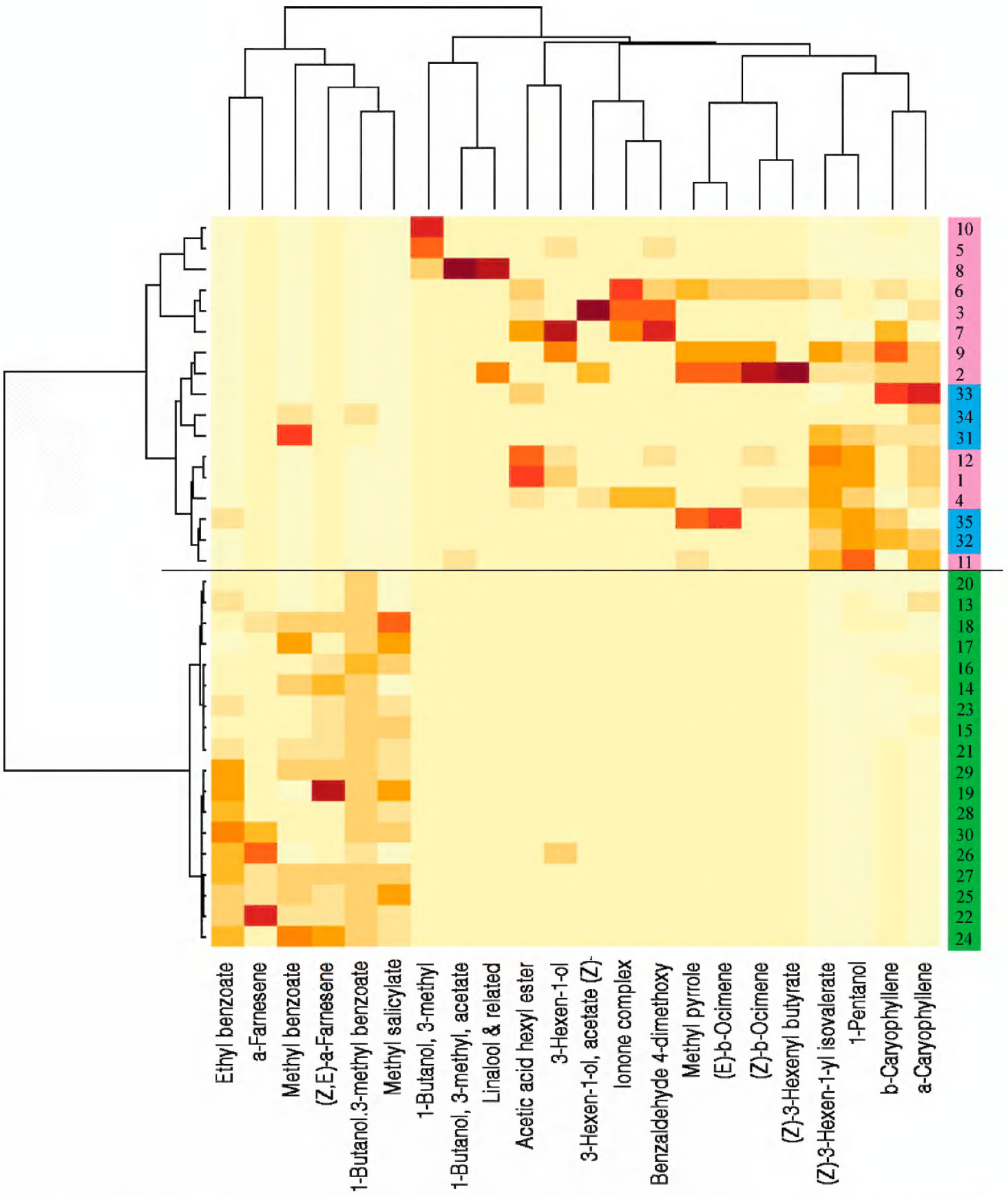


**Figure 3.** VOCs similarity histogram of *Rhamnus alaternus* (violet), *Rh.* ×*bermejoi* (blue) and *Rh. ludovici-salvatoris* (green). Colour intensity is related to the level of presence of a component in each sample.

such as in the genus *Clusia* (Nogueira et al. 2001), *Nicotiana* (Raguso et al. 2006), *Lilium* (Kong et al. 2012) or *Artemisia* (Jaeger et al. 2016). The results obtained in *Rh. alaternus* and *Rh. ludovici-salvatoris* and their hybrids were consistent with these observations.

The main differences between *Rh. alaternus* and *Rh. ludovici-salvatoris* were found in the types of terpenoids and benzoids emitted, as well as in their emission rates (Suppl. material 2). Thus, among the terpenoids, *R. alaternus* exhibited the emission

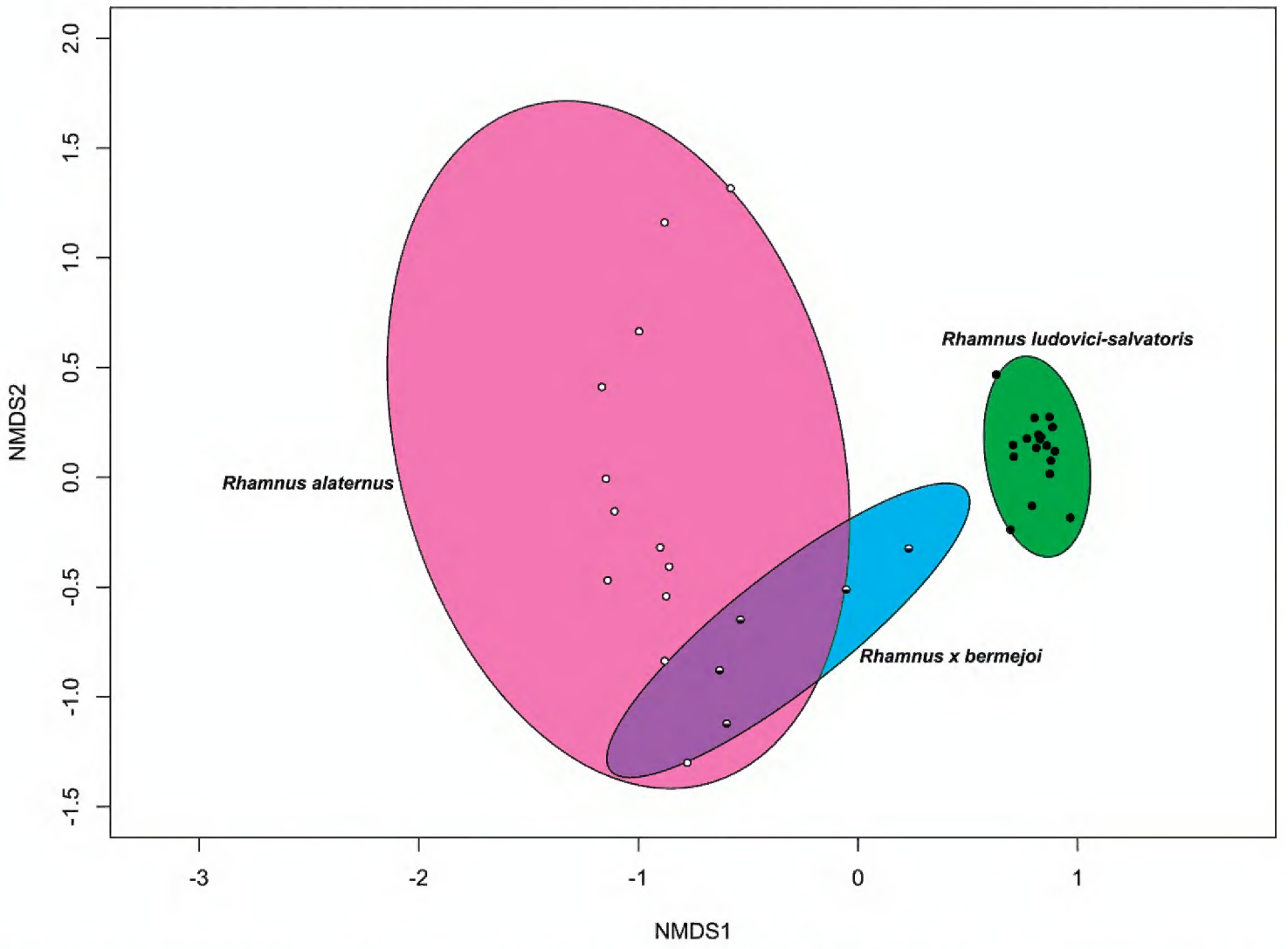


**Figure 4.** Non-metric multidimensional scaling ordination (NMDS) of floral scent profiles for *Rhamnus alaternus* (violet and empty circles), *Rhamnus ×bermejoi* (blue and half black circles) and *Rhamnus ludovici-salvatoris* (green and black points).

**Table 1.** Results of SIMPER analysis indicate the cumulative contributions of most influential compounds. AvA: Average in *Rhamnus alaternus*; AvB: Average in *Rhamnus ludovici-salvatoris*; Cumsum: cumulative contribution (in%).

| | AvA | AvB | Cumsum |
|---|---|---|---|
| Methyl salicylate | 0.00 | 82.32 | 44.4 |
| 3-Hexen-1-ol, acetate (Z)- | 24.88 | 0.64 | 57.6 |
| Ionone complex | 16.09 | 0.00 | 66.4 |
| b-Caryophyllene | 15.18 | 0.00 | 74.6 |
| Methyl benzoate | 0.00 | 9.25 | 79.6 |
| Linalool and related | 9.11 | 0.00 | 84.4 |
| Benzaldehyde 4-dimethoxy | 5.23 | 0.00 | 87.1 |
| (Z)-b-Ocimene | 4.69 | 0.00 | 89.7 |
| 1-Butanol, 3 methyl, acetate | 4.26 | 0.54 | 92.0 |

of monoterpenes ((Z)- and (E)-β-ocimene and linalool and related compounds) and ionones; all these products are absent in *Rh. ludovici-salvatoris*. There were also different types of sesquiterpenes, farnesenes being characteristic of *Rh. ludovici-salvatoris* and caryophyllenes being characteristic of *Rh. alaternus*. On the other hand, the uniformity and

richness of benzoids in *Rh. ludovici-salvatoris* (specifically, methyl salicylate and methyl benzoate) were very prevalent. This finding contrasts with its absence in *Rh. alaternus*, because in this species, only the rare presence of benzaldehyde 4-dimethoxy has been recognised. Methyl salicylate (83.3%) and methyl benzoate (9.2%) constitude the primary components of the floral aroma in *Rh. ludovici-salvatoris*. These compounds are also present in hybrids but notably absent in *Rh. alaternus*. Both esters are biosynthesised by the action of carboxyl methyltransferases and have been associated with pollinator attraction (Negre et al. 2003), but they also play an important role as aerial signals involved in inter- and intra-plant communication to activate disease (Shulaev et al. 1997) and, as also seems plausible in this case, signalling an abiotic stress situation for this species (Munné-Bosch and Peñuelas 2003; Karl et al. 2008; Liu et al. 2018; Gondor et al. 2022).

The high intra-population heterogeneity of chemical compounds of *Rh. alaternus* was evidenced by the fact that not all differential compounds were found in all the plants; however, some of them were always present. This observation stands in contrast with *Rh. ludovici-salvatoris*, which has a high chemical similarity with all plants, as evidenced by the presence of its main differential compounds, methyl salicylate and methyl benzoate. These chemical diversity profiles followed a pattern similar to that of genetic diversity (Ferriol et al. 2009; Hauenschild et al. 2016), which recognizes much higher levels of genetic variation in the populations of *Rh. alaternus* than in those of endemic *Rh. ludovici-salvatoris*.

The qualitative and quantitative patterns of VOC expression in hybrids are heterogeneous (López-Caamal 2014). In some species, hybridization could lead to the production of new secondary metabolites that are not present in parental species, due to the obstruction of biosynthetic pathways (Vereecken et al. 2010; Kong et al. 2012). In other hybrid species, highly variable proportions of several compounds are present. In some, the emission rates of some of these may be interpreted as transgressive with the parents; however, they are not always consistently intermediate. In others, only the emission of specific compounds was intermediate (Bischohff et al. 2014). In general, whenever hybridization occurs, a large qualitative variation in secondary metabolites is expected. In the studied hybrids, compounds shared with parental strains were produced at different levels, but no new products were recognized (Suppl. material 2). In the amounts of shared compounds, there was a clear tendency to be more like *Rh. alaternus* (Fig. 4).

Both personal observations and those of Canale et al. (2016) showed that the main pollinators of the two *Rhamnus* species were Hymenoptera (mainly *Apis mellifera* Linnaseus, 1758). This finding was consistent with the fact that the two groups of BVOCs that are dominant in the flowers of the two species of *Rhamnus* attract honey bees: terpenoids for *Rh. alaternus* (Dobson 2006; Twidle et al. 2015) and benzoids for *Rh. ludovici-salvatoris* (Negre et al. 2003; Mallinger et al. 2011; Dötterl et al. 2014). Thus, this could be the pollination vector that mediates the hybridization process.

When the diversity of BVOCs is greater in hybrids than in the parentals, their heterogeneity and progression are encouraged, since this could help in attracting more pollinator species (Ayasse et al. 2011), breaking the reproductive isolation of hybrids. However, this increase does not occur in the studied hybrids; therefore, their VOC content, as in their parent *R. ludovici-salvatoris*, was not a factor that favours pollination and reproduction.

Under these conditions, compared to *Rh. alaternus*, lower photosynthetic efficiency of *Rh. ludovici-salvatoris* and its hybrids in the current climatic Mediterranean circumstances (Yll et al. 1997; Gulias et al. 2002) would be, together with the inability of regrowth, the main cause that would explain both the progressive decrease in the populations of this species as well as the rarity of hybrids and the relictual character of the current population of *Rh. ludovici-salvatoris* and its hybrids in Minorca (Fraga and Bermejo 2008).

## Conclusions

The analysis of the floral volatile compounds in the studied taxa reveals both qualitative and quantitative differences in the chemical profile of *R. alaternus* and *Rh. ludovici-salvatoris*. Among them, the discriminatory nature of various compounds stands out, such as methyl salicylate and methyl benzoate (present in *Rh. ludovici-salvatoris* and absent in *Rh. alaternus*), linalool and related compounds, ionones, β-caryophyllene, 4-dimethoxy benzaldehyde, and (Z)-β-ocimene (present in *Rh. alaternus* and absent in *Rh. ludovici-salvatoris*). In contrast, the chemical profile does not allow differentiation between *Rh. alaternus* and the hybrid *Rh.* ×*bermejoi*. According to these results, it is suggested that floral volatile compounds could be an effective discriminatory trait among species in *Rh.* sect. *Alaternus*. This finding should be confirmed through the study of plants from other populations of *Rh. alaternus*, as well as samples from *Rh. lojaconoi* and the Macaronesian endemic species *Rh. glandulosa* and *Rh. integrifolia*.

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## References

Adams RP (2007) Identification of Essential oil Components by Gas Chromatography/ MassSpectroscopy. Carol Stream, IL. Allured Publishing Corporation.

Ahmad R, Baharum SN, Bunawan H, Lee M, Mohd Noor N, Rohani ER, Ilias N, Zin NM (2014) Volatile profiling of aromatic traditional medicinal plant, *Polygonum* minus in different tissues and Its biological activities. Molecules 19: 19220–19242. https://doi.org/10.3390/molecules191119220

Aysse M, Stökl J, Francke W (2011) Chemical ecology and pollinator-driven speciation in sexuallly deceptive orchids. Phytochemistry 72: 1667–1677. https://doi.org/10.1016/j.phytochem.2011.03.023

Bischoff M, Jürgens A, Campbell DR (2014) Floral scent in natural hybrids of *Ipomopsis* (Polemoniaceae) and their parental species. Annals of Botany 113: 533–544. https://doi.org/10.1093/aob/mct279

Bouwmeester H, Schuurink RC, Bleeker PM, Schiestl F.(2019) The role of volatiles in plant communication. Plant Journal 100(5): 892–907. https://doi.org/10.1111/tpj.14496

Canale A, Benvenuti S, Raspi A, Benelli G (2016) Insect pollinators of the late winter flowering *Rhamnus alaternus* L., a candidate for honeybee-friendly scrubland spots in intensively managed agricultural areas. Plant Biosystems 150: 611–615. https://doi.org/10.1080/11263504.2014.993742

Cardona MA, Contandriopoulos J (1979) Endemism and evolution in the islands of the western Mediterranean. In: Bramwell D (Ed.) Plant and islands. Academic Press, London, UK, 133–169.

Carta A, Flamini G, Cioni PL, Pistelli L, Peruzzi L (2015) Flower bouquet variation in four species of *Crocus* ser. *Verni*. Journal of Chemical Ecology 41: 105–110. https://doi.org/10.1007/s10886-014-0541-y

Cheng D, Vrieling K, Klinkhamer PG (2011) The effect of hybridization on secondary metabolites and herbivore resistance: implications for the evolution of chemical diversity in plants. Phytochemistry Reviews 10: 107–117. https://doi.org/10.1007/s11101-010-9194-9

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Clarke KR, Gorley RN, Somerfield PJ, Warwick RM (2014) Change in marine communities: an approach to statistical analysis and interpretation, 3nd edn. PRIMER-E Ltd, Plymouth, 1–262.

Cronquist A (1977) On the taxonomic significance of secondary metabolites in Angiosperms. In: Kubitzki K (Ed.) Flowering Plants. Plant Systematics and Evolution / Entwicklungsgeschichte und Systematik der Pflanzen, vol 1. Springer, Vienna. Flowering Plants, 179–189. https://doi.org/10.1007/978-3-7091-7076-2_12

Dobson HEM (2006) Relationship between floral fragrance composition and type of pollinators. In: Duradeva N, Pichersky E (Eds) Biology of floral scent. CRC Taylor & Francis, 147–198. https://doi.org/10.1201/9781420004007-8

Dötterl S, Glück U, Jürgens A, Woodring J, Aas G (2014) Floral reward, advertisement and attractiveness to honey bees in dioecious Salix caprea. PLoS ONE 9(3): e93421. https://doi.org/10.1371/journal.pone.0093421

El-Sayed AM (2023) The Pherobase: Data base of Insect Pheromones and Semiochemicals. http://www.pherobase.com

Ferriol M, Llorens L, Gil L, Boira H (2009) Influence of phenological barriers and habitat differentiation on the population genetic structure of the Balearic endemic *Rhamnus ludovici-salvatoris* Chodat and *Rh. alaternus* L. Plant Systematics and Evolution 277: 105–116. https://doi.org/10.1007/s00606-008-0110-3

Feulner M, Schuhwerk F, Dötterl S (2011) Taxonomical value of inflorescence scent in *Hieracium* s. str. Biochemical Systematics and Ecology 39: 732–743. https://doi.org/10.1016/j.bse.2011.06.012

Fraga P, Bermejo A (2008) Notes i contribucions al coneixement de la flora de Menorca (IX): característiques i estat de conservació de la població menorquina de *Rhamnus ludovici-salvatoris* Chodat. Bolletí de la Societat d'Història Natural de les Balears 51: 17–32.

Fraga P, Rosselló JA (2008) *Rhamnus* ×*bermejoi*, a new wild hybrid between *Rh. alaternus* and *Rh. ludovici-salvatoris*. Flora Mont 40: 47–79.

Friberg M, Schwind C, Raguso R, Thompson JN (2013) Extreme divergence in floral scent among woodland star species (*Lithophragma* spp.) pollinated by floral parasites. Annals of Botany 111: 539–550. https://doi.org/10.1093/aob/mct007

Georgescu L, Stefanakis MK, Kokkini S, Katerinopoulos HE, Pirintsos SA (2015) Chemical and genetic characterization of *Phlomis* species and wild hybrids in Crete. Phytochemistry 122: 91–102. https://doi.org/10.1016/j.phytochem.2015.11.007

Gil-ad NL, Reznicek AA (1997) Evidence for hybridation of two Old Word *Rhamnus* species -*R. cathartica* and *R. utilis* (Rhamnaceae)- in the New World. Rhodora 99: 1–22.

GISD [Global Invasive Species Database] (2018) Species profile: *Rhamnus alaternus*. http://www.iucngisd.org/gisd/species.php?sc=893 [Downloaded on 10-09-2018]

Gögler J, Stökl J, Sramkova A, Twele R, Francke W, Cozzolino S, Cortis P, Scrugli A, Ayasse M (2009) Menage a trios: two endemic species of deceptive orchids and one pollinator species. Evolution 63: 2222–2234. https://doi.org/10.1111/j.1558-5646.2009.00712.x

Gondor OK, Pál M, Janda T, Szalai G (2022) The role of methyl salicylate in plant growth under stress conditions. Journal of Plant Physiology 277: 153809. https://doi.org/10.1016/j.jplph.2022.153809

Goswami P, Chauhan A, Verma RS, Padalia RC (2016) Chemical constituents of floral volatiles of *Plumeria rubra* L. from India. Medicinal and Aromatic Plants Research Journals S3: 005. https://doi.org/10.4172/2167-0412.S3-005

Gulias J, Flexas J, Abadia A, Medrano H (2002) Photosynthetic responses to water deficit in six mediterranean sclerophyll species: possible factors explaining the deckining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. Tree Physiology 22: 687–697. https://doi.org/10.1093/treephys/22.10.687

Hadacek F (2002) Secondary metabolites as plant traits: current assessment and future perspectives. Critical Reviews in Plant Sciences 21: 273–322. https://doi.org/10.1080/0735-260291044269

Hauenschild F, Favre A, Gerardo A, Salazar GA, Muellner-Riehl AN (2016) Analysis of the cosmopolitan buckthorn genera *Frangula* and *Rhamnus* s.l. supports the description of a new genus, Ventia. Taxon 65: 65–78. https://doi.org/10.12705/651.5

Hegnauer R (1986a) Phytochemistry and plant taxonomy-an essay on the chemotaxonomy of higher plants. Phytochemistry 25: 1519–1535. https://doi.org/10.1016/S0031-9422(00)81204-2

Hegnauer R (1986b) Comparative phytochemistry and plant taxonomy. Gior Botany Italia 120: 15–26. https://doi.org/10.1080/11263508609428018

Henning T, Raab-Straube E von (2016): Rhamnaceae. In: Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity. http://ww2.bgbm.org/EuroPlusMed/ [Accessed 2023]

Jaeger DM, Runyon JB, Richardson BA (2016) Signals of speciation: volatile organic compounds resolve closely related sagebrush taxa, suggesting their importance in evolution. New Phytologist 211: 1393–1401. https://doi.org/10.1111/nph.13982

Karl T, Guenther A, Turnipseed A, Patton EG, Jardine K (2008) Chemical sensing of plant stress at the ecosystem scale. Biogeosciences 5: 1287–1294. https://doi.org/10.5194/bg-5-1287-2008

Kong Y, Sun M, Pan H, Zhang Q (2012) Composition and emission rhythm of floral scent volatiles from eight lily cut flowers. Journal of the American Society for Horticultural Science 137: 376–382. https://doi.org/10.21273/JASHS.137.6.376

Kurylo JS, Knight KS, Stewart JR, Endress AG (2007) *Rhamnus cathartica*: native and naturalized distribution and habitat preferences. The Journal of the Torrey Botanical Society 143: 420–430. https://doi.org/10.3159/1095-5674(2007)134[420:RCNAND]2.0.CO;2

Levin RA, McDade L, Raguso RA (2003) The systematic utility of floral and vegetative fragrance in two genera of Nyctaginaceae. Systematic Biology 52: 334–351. https://doi.org/10.1080/10635150390196975

Liu B, Kaurilind E, Jiang Y, Niinemets Ü (2018) Methyl salicylate differently affects benzenoid and terpenoid volatile emissions in *Betula pendula*. Tree Physiology 38(10): 1513–1525. https://doi.org/10.1093/treephys/tpy050

Llorens L (1979) Nueva contribución al conocimiento de la flora balear. Mediterranea 3: 101–122. https://doi.org/10.14198/MDTRRA1979.3.04

López-Caamal A, Tovar-Sánchez E (2014) Genetic, morphological, and chemical patterns of plant hybridization. Revista Chilena de Historia Natural 87: 16. https://doi.org/10.1186/s40693-014-0016-0

López-González G (2006) Los árboles y arbustos de la Península ibérica e Islas Baleares. Mundi-Prensa, Barcelona, 533–536.

Mallinger RE, Hogg DB, Gratton C (2011) Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in Soybean Agroecosystems. Journal of Economic Entomology 104: 115–124. https://doi.org/10.1603/EC10253

Martinez-Arbizu P (2020) pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.4. https://github.com/pmartinezarbizu/pairwiseAdonis

Meekijjaroenroj A, Bessière JM, Anstett MC (2007) Chemistry of floral scents in four *Licuala* species (Arecaceae). Flavour and Fragrance Journal 22: 300–310. https://doi.org/10.1002/ffj.1797

Munné-Bosch S, Peñuelas J (2003) Photo-and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. Planta 217: 758–766. https://doi.org/10.1007/s00425-003-1037-0

Muyt A (2001) Bush Invaders of South-east Australia. RG and FJ Richardson Meredith, Victoria, Australia, 213–214.

Negre F, Kish CM, Boatright J, Underwood B, Shibuya K, Wagner C, Clark DG, Dudareva N (2003) Regulation of methylbenzoate emission after pollination in snapdragon and *Petunia* flowers. The Plant Cell 15: 2992–3006. https://doi.org/10.1105/tpc.016766

Nogueira PCL, Bittrich V, Shepherd GJ, Lopes AV, Marsaioli AJ (2001) The ecological and taxonomic importance of flower volatiles of *Clusia* species (Guttiferae). Phytochemistry 56: 443–452. https://doi.org/10.1016/S0031-9422(00)00213-2

Nwafor FI, Orabueze IC (2018) Role of phytochemistry in plant classification: Phytochemotaxonomy. In: Egbuna C, Ifemeje JC, Udedi SC, Kumar S (Eds) Phytochemistry: Fundamentals, Modern Techniques, and Applications vol 1, Apple Academic Press, 198–224.

Oksanen AJ, Blanchet FG, Friendly M, Kindt R, Legendre P, Mcglinn D, Minchin PR, Hara RBO, Simpson GL, Solymos P, Stevens MHH, Szoecs (2019) E. vegan:

community ecology package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Orians CM (2000) The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant-herbivore interactions. American Journal of Botany 87: 1749–1756. https://doi.org/10.2307/2656824

Peruzzi L, Roma-Marzio F, Dolci D, Flamini G, Braca A, De Leo M (2019) Phytochemical data parallel morpho-colorimetric variation in *Polygala flavescens* DC. Plant Biosystems 153(6): 817–834. https://doi.org/10.1080/11263504.2018.1549615

Raguso RA, Schlumpberger BO, Kaczorowski RL, Holtsford TP (2006) Phylogenetic fragrance patterns in *Nicotiana* sections Alatae and Suaveolentes. Phytochemistry 67: 1931–1942. https://doi.org/10.1016/j.phytochem.2006.05.038

Rivas-Martínez S, Pizarro JM (2011) Taxonomical system advance to *Rhamnus* L. & *Frangula* Mill. (Rhamnaceae) of Iberian Peninsula and Balearic Islands. International Journal of Geobotanical Research 1: 55–78. https://doi.org/10.5616/ijgr110004

Rivas-Martínez S, Pizarro JM (2013) *Rhamnus* L. In: Muñoz Garmendia F, Navarro C, Quintanar A, Buira A (Eds) Flora Iberica 9, Real Jardín Botánico, CSIC, Madrid, 36–44.

Roma-Marzio F, Najar B, Alessandri J, Pistelli L, Peruzzi l (2017) Taxonomy of prickly juniper (*Juniperus oxycedrus* group): A phytochemical–morphometric combined approach at the contact zone of two cryptospecies. Phytochemistry 141: 48–60. https://doi.org/10.1016/j.phytochem.2017.05.008

Seigler DS (1981) Secondary metabolites and plant systematics. In: Conn EE (Ed.) The biochemistry of plants. Secondary plant products vol 7, Academic Press, 139–171. https://doi.org/10.1016/B978-0-12-675407-0.50012-9

Shulaev V, Silverman P, Raskin I (1997) Airborne signalling by methyl salicylate in plant pathogen resistance. Nature 385: 718–721 https://doi.org/10.1038/385718a0

Singh R (2016) Chemotaxonomy: A Tool for Plant Classification. Journal of Medicinal Plants Studies 4(2): 90–93.

Stocki M, Banaszczak P, Stocka N, Borowik T, Zapora E, Isidorov V (2020) Taxonomic implications of volatile secondary metabolites emitted from birch (*Betula* L.) buds. Biochemical Systematics and Ecology 92: 104132. https://doi.org/10.1016/j.bse.2020.104132

Tomas J, Cardona C, Ferriol P, Llorens L, Gil L (2022) Floral traits and reproductive biology of two Mediterranean species of *Clematis*, asynchronous and sympatric, are key food sources for pollinator survival. South African Journal of Botany 151: 85–94. https://doi.org/10.1016/j.sajb.2022.09.027

Tóth P, Undas AK, Verstappen F, Bouwmeester H (2016) Floral Volatiles in Parasitic Plants of the Orobanchaceae. Ecological and Taxonomic Implications. Frontiers in Plant Science 7: 312. https://doi.org/10.3389/fpls.2016.00312

Tundis R, Peruzzi L, Menichini F (2014) Phytochemical and biological studies of *Stachys* species in relation to chemotaxonomy: a review. Phytochemistry 102: 7–39. https://doi.org/10.1016/j.phytochem.2014.01.023

Twidle AM, Mas F, Harper AR, Horner RM, Weish TJ, Suckling DM (2015) Kiwifruit flower odor perception and recognition by honey bees, *Apis mellifera*. Journal of Agricultural and Food Chemistry 63: 5597–5602. https://doi.org/10.1021/acs.jafc.5b01165

Vereecken NJ, Cozzolino S, Schiestl FP (2010) Hybrid floral scent novelty drives pollinator shift in sexually deceptive orchids. BMC Evolutionary Biology 10: 103. https://doi.org/10.1186/1471-2148-10-103

Whitehead MR, Peakall R (2009) Integrating floral scent, pollination ecology and population genetics. Functional Ecology 23: 863–874. https://doi.org/10.1111/j.1365-2435.2009.01620.x

Yang L, Ren J, Wang Y (2014) Chemical investigation of volatiles emitted from flowers of three varieties of Damask rose cultivated in Beijing. Horticulture Environment and Biotechnology 55: 524–530. https://doi.org/10.1007/s13580-014-0176-5

Yll E, Pérez-Obiol R, Pantaleon-Cano J, Roure JM (1997) Palynological evidence for climatic change and human activity during the Holocene on Minorca (Balearic Islands). Quaternary Research 48: 339–347. https://doi.org/10.1006/qres.1997.1925

## Supplementary material 1

**Geographical location and sexuality of *Rhamnus* sampled plants**

Authors: Leonardo Llorens, Pere Ferriol, Joan Tomàs, María Trinidad García, Lorenzo Gil

Data type: xlsx

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## Supplementary material 2

**Floral scent chemistry**

Authors: Leonardo Llorens, Pere Ferriol, Joan Tomàs, María Trinidad García, Lorenzo Gil

Data type: docx

Explanation note: Floral scent chemistry of *Rhamnus alaternus* (blue), *R. ludovici-salvatoris* (yellow) and hybrids (green). For each species, compounds with presence in >10% of samples.

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